results reveal biophysical roles of adhesion and cytoskeletal tension in regulating mRNAs to protrusions.

3169-Pos Board B599
Mechanics of Optic Vesicle Morphogenesis in the Chick Embryo
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In the vertebrate embryo, the eyes are initially bulges that grow outward from the sides of the primitive forebrain. As these optic vesicles (OVs) elongate, they come into contact with the surrounding surface ectoderm (SE), and both layers then invaginate to create the optic cup (prospective retina) and lens vesicle. The initial shaping of the OVs sets the stage for these later events. To explore the mechanical factors involved in shaping the OVs, we used experiments on chick embryos along with computational models. First, mechanical dissections were used to remove the SE. Our analysis of OV shapes suggests that the SE exerts asymmetric loads that cause the OVs to flatten and shear caudally during the earliest stages of eye development and later to bend in the caudal and dorsal directions. These deformations cause the initially spherical OVs to become pear-shaped. Exposure to the myosin II inhibitor blebbistatin reduced these effects, suggesting that cytoskeletal contraction controls OV shape by regulating tension in the SE. To test the physical plausibility of these interpretations, we developed finite-element models of the forebrain, including frictionless contact between the SE and OVs. With differential growth included in the OVs, these models were used to simulate each experiment (control, SE removed, no contraction). For each case, the predicted shape of the OV agrees reasonably well with experiments. Our results support this idea that a combination of differential growth in the OV and external pressure exerted by the SE are sufficient to cause the global changes in OV shape observed during the earliest stages of eye development.

3170-Pos Board B600
Cell-Free Expression Systems: From Gene Circuits to Self-Assembly Processes in a Test Tube
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Cell-free transcription-translation (TX-TL) systems are becoming powerful platforms to construct complex biological systems in vitro through the expression of DNA programs. Considerable efforts have been made to improve those systems in the past decade. Our laboratory has developed the most efficient and versatile in vitro E. coli TX-TL system to express synthetic or natural DNA programs encoding for self-assembly processes. Either in test tube reactions, in emulsion droplets or in liposomes, cell-free TX-TL reactions are now used for research studies ranging from elementary gene circuits to minimal cells.
I will present our custom-made cell-free TX-TL system, its current capabilities and limitations. Recently we have shown that entire phages can be synthesized in cell-free TX-TL reactions from their genomes. The bacteriophage T7, composed of about 60 genes, is entirely synthesized in a single test tube reaction from its 40 kbp genomic DNA. Replication of the T7 DNA instructions occurs concurrently with phage expression and self-assembly. The phage phiX174 composed of a dozen of genes can also be synthesized from its genome. Encapsulated inside cell-sized phospholipid liposomes, the TX-TL system is used to construct a prototype of minimal cell. I will present this cell-free synthetic biology platform, our last experiments and how this system can be used to study the relationship between information and self-organization.

3171-Pos Board B601
Increasing Targeting and Efficacy of Anti-Tumor Antibody
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In cancer research one of the most difficult aspects is finding a treatment that selectively targets diseased cells without harming healthy cells in their proximity. This lead to inquiry about the utilization of a patients own immune system. It has been found that cytotoxic immune cells infiltrate tumor masses but are inactivated by intercellular signals. Our lab sought to construct an antibody that would bind specifically tumor cells with a high affinity, as well as activating infiltrating T lymphocytes. Previously, our lab identified a antigen binding fragment (Fab) that localized to tumors and bound an E75-MHC receptor found on their cell surface with nanomolar affinity. Using SPR studies the half-life of this binding was found to be less than a 2 minutes and exhibited a problematic duration in vivo. Our objective was to design, collect and test the avidity of an antibody with a bivalent Fab display. In addition to the Fab elements, mutations were made in the constant region (Fc) of the antibody to include an anti-CD3 small chain variable fragment, enabling binding to the CD3 glycoprotein found on the surface of T cells, and a 6-histidine, to be used during purification. Two Escherichia Coli expression methods were attempted; experimenting with phosphate depletion and ITPG induced promoters. Neither provided significant yield, so a mammalian expression model was chosen. The mammalian model produced a higher yield using transiently transfected kidney cells. The protein was purified using fast protein liquid chromatography, running through two columns. A Protein A column selected for Ig folds, isolating all formed antibodies. A HisTalon column selected for histidine, removing formed protein with double CD3 reactive Fc elements, which cause systemic immune response. Unfortunately errors in our MHC receptor expression prevented the project from completing our avidity studies.

3172-Pos Board B602
Exploring Biologically based Malnourishment through a Gut-On-A-Chip Approach
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Malnourishment results in a cycle that degrades the ability to effectively absorb nutrients even after adequate food has been consumed due to a disruption of gut homeostasis. We aim to better understand impaired nutrient absorption in malnourished individuals through a microfluidic based lab-on-a-chip approach. We are developing a malnourished model of an intestine into existing gut-on-a-chip device design fabricated through soft lithography techniques. In order to develop a more physiologically relevant model, we have studied the effects of different components of bacteria on the mechanical properties of the gut cells. Whole bacteria, spheroplasts, and the endotoxin lipopolysaccharide (LPS) were all introduced to the gut cell layer to determine their adverse effects on the stability of the cells. We characterized this malnourished model by studying the tight junction integrity along with cytoskeletal organization of the microvilli in both healthy and malnourished models. Additionally, we have looked at the permeability of the monolayer as an indicator of epithelial integrity. Our work would be useful in many areas including evaluating malnourished nutritional absorption in vitro as well as demonstrating the response of malnourished cells in a more physiologically relevant assay.

3173-Pos Board B603
Developing a Microfluidic Device for Adenoviral Transfection of Pancreatic Islets
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Diabetes is a global epidemic that is associated with the deterioration of pancreatic beta-cell mass and function. Beta-cell function within the islet has previously been studied ex vivo and the role of specific proteins is commonly investigated with overexpression models using viral transfection. However, poor penetration and reduced transfection efficiency limits the application of this technology in whole ex vivo islets. To improve transfection efficiency and maintain islet structure, we aim to develop a method to bring viral particles deeper into the tissue. Improving upon a previous microfluidic device design featuring hydrodynamic traps (nozzles) in series, a spiral-shaped microfluidic device with parallel nozzles was created to trap, transfect and deliver nutrients to islets. The device was engineered to provide sufficient resistance and pressure drop along the nozzles, allowing for homogeneous transfection throughout captured islets. Computer-generated models were used to optimize our design parameters to minimize pressure drop variations along with shear induced damage of islets. Through quantitative fluorescence microscopy, we have measured the media exchange rate through islets and have determined a range of operating flow-rates. We have also shown successful culturing of the loaded islets in our microfluidic device. Beta-cell-specific transfection in whole islets with desired genes will facilitate the investigation of islets in a more physiologically relevant environment. Therefore, we have developed a beta-cell specific shuttle vector in order to deliver fluorescent protein-tagged genes via adenoviral particles. Currently viral penetration efficiency is being tested in this device through fluorescence microscopy and western immunoblotting. We aim to create a multi-purpose platform that is not limited to the study of beta-cell biology in islets, but may be translated to the investigation of other tissue models and diseases.